Analysis of Sulfa Drugs by HPLC

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Abstract

The sulfa drugs analyzed here all contain a sulfanilamide substructure as active group. They are used against infections by salmonellae, streptococci, staphylococci and coli bacteria.

The HPLC method described here separates the five common sulfa drugs sulfanilamide, sulfadiazine, sulfathiazole, sulfamerazine and sulfamethazine using gradient analysis on a reversed phase column and UV detection. The autosampler temperature was set to 4 ºC to avoid decomposition of the samples.

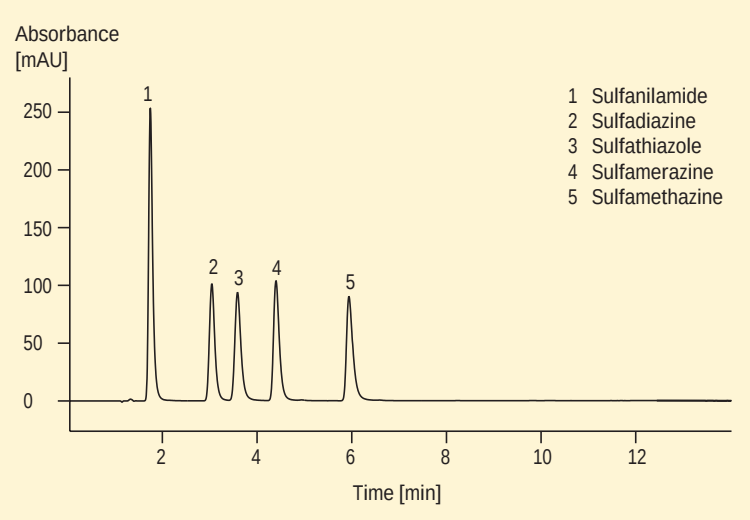

Figure 1
Analysis of five sulfa drugs

Conditions

Column
4 x 125 mm ODS Hypersil 5 µm
Mobile phase
A = water
B = acetonitrile
Flow rate
1.0 ml/min
Gradient
10 % B to 25 % B in 10 min
Column wash
25 % B to 10 % B in 2 min
UV detector
variable wavelength detector
254 nm, standard cell
Column compartment temperature
50 °C
Stop time
12 min
Post time
5 min
Injection volume
5 µl

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The performance of the HPLC method is shown in the table below.

Compound	LOD for S/N=2 (mg/l)*	Precision of RT (RSD of 10 runs) (100 mg/l)*	Precision of Area (RSD of 10 runs) (100 mg/l)*
Sulfanilamide	0.1	0.12	0.31
Sulfadiazine	0.2	0.06	0.28
Sulfathiazole	0.2	0.06	0.29
Sulfamerazine	0.2	0.05	0.30
Sulfamethazine	0.2	0.05	0.30

* Injection volume: 5 µl

The HPLC method presented here shows an easy but reliable and precise analysis of the sulfa drugs sulfanilamide, sulfadiazine, sulfathiazole, sulfamerazine and sulfamethazine. The values for LOD, precision of RT and precision of area show the good performance of the analysis.

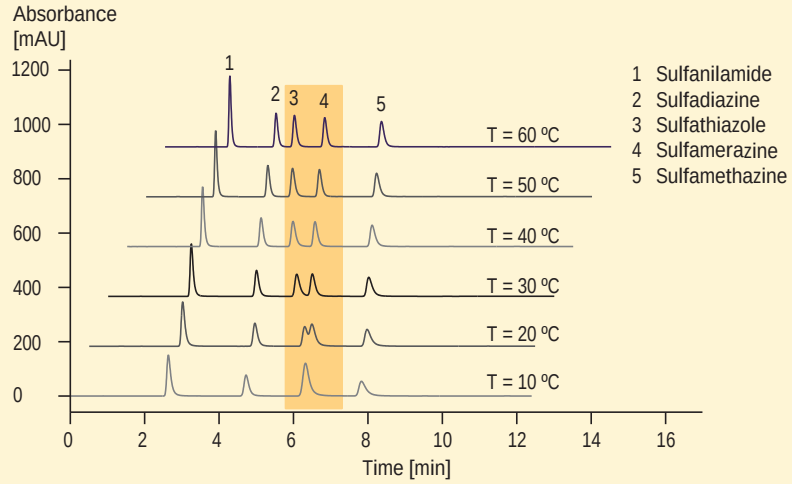

Figure 2
Separation of sulfathiazole and sulfamerazine influenced by column temperature

Note:
Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance. It is essential to use a thermostatted column compartment, for example, the Agilent 1100 Series thermostatted column compartment, to separate the compounds sulfathiazole and sulfamerazine, as shown in figure 2. To avoid sample decomposition, it is necessary to use a thermostatted autosampler, for example, the Agilent 1100 Series thermostatted autosampler.

Equipment

Agilent 1100 Series
- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell, 10-mm path length, 13-µl cell volume

Alternative:
- Vacuum degasser
- Binary pump
- Diode-array detector, standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

Columns
- Hypersil ODS, 5 µm, 4 x 125 mm (Agilent part number 792618-564)
- *Recommended:* Guard cartridges Hypersil ODS, 5 µm, 4 x 4 mm (Agilent part number 7992618-504, 4/pk)

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